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**Crystallographic analysis of 6-pyruvoyl tetrahydropterin synthase from E.coli (ePTPS)**

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6-pyruvoyl tetrahydropterin synthase (ePTPS) is the second enzyme in the biosynthesis of tetrahydrobiopterin (BH4). It catalyzes generally the conversion of dihydropterin triphosphate (HTP2) to 6-pyruvoyl tetrahydropterin (PTP) in a Zn²⁺ and Mg²⁺-dependent reaction without the consumption of an external reducing agent. Comparing with the animal enzymes, PTPs from E. coli (ePTPS) possesses another much stronger catalytic activity to cleave the side chain of the pterin substrate rather than the conversion from H2PTP to PTP. To examine the extraordinary enzyme activity of ePTPS, we have expressed a soluble ePTPS in E. coli and purified by nickel-agarose affinity, ion exchange and gel filtration chromatography. ePTPS exists as a trimer in solution. For structural analysis, ePTPS was crystallized in 0.02 M MgCl₂, 0.1 M HEPES pH 7.5, 22 % polyacrylic acid 5100 salt by using hanging drop vapor diffusion method. Diffraction data were collected to 2.9 Å resolution using synchrotron radiation. ePTPS crystals belong to hexagonal space group P321, with unit-cell parameters a =112.589 Å, b= 125.589 Å, c= 68.818 Å, α = β = γ = 90°, β = 120° and contains 2 molecules in the asymmetric unit. The structure of ePTPS was determined by molecular replacement. In the ePTPS crystal structure, each monomer (121 residues) folds into a single domain of two α-helixes and four β-sheets. In the crystal, ePTPS forms a hexamer made by two closely interacting trimers generated by the crystallographic symmetry mates of each molecule in the asymmetric unit.

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**Cristallization and preliminary X-ray crystallographic analysis of enoyl-ACP reductase III (FabL) from Bacillus subtilis**

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Enoyl-[acyl-carrier-protein] (ACP) reductase (ENR) is a key enzyme in type II fatty-acid synthase that catalyzes the last step in each elongation cycle. It has been considered as an antibiotic target, since it is an essential enzyme in bacteria. However, recent studies indicate some pathogens have more than one ENR. Bacillus subtilis is reported to have two ENRs, namely FabI and FabL. While BsFabI is similar to other FabIs, BsFabL shows very little sequence similarity and is NADPH dependent instead of NADH as in FabI. In order to understand these differences on a structural basis we have cloned and overexpressed BsFabL, and crystallized it. The crystal belongs to space group P2₁2₁2₁ with unit cell parameters of a = b = c = 139.56 Å, α = 62.75 Å, β = γ = 90°, z = 120° with one molecule of FabL in the asymmetric unit. Data were collected using synchrotron radiation (beamline 4A at the Pohang Light Source, Korea). The crystal diffractiond to 2.5 Å resolution, spacegroup symmetry by the Functional Proteomics Center, the 21C Frontier Research & Development Program of the Korea Ministry of Science and Technology and the KIST grant.

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**Crystal structure of the GluR0 ligand-binding core from Nostoc punctiforme in complex with L-glutamate reveals a Novel subunit interface**

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GluR0 from Nostoc punctiforme (NpGluR0) is another bacterial homolog of iGluRs dimer interface. In the ePTPS crystal structure, each monomer (121 residues) folds into a single domain of two α-helixes and four β-sheets. In the crystal, ePTPS forms a hexamer made by two closely interacting trimers generated by the crystallographic symmetry mates of each molecule in the asymmetric unit.

**A-100**

**Crystal structure of human nucleophosmin-core reveals plasticity of the pentamer- pentamer interface**

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Human nucleophosmin (NPM; also known as B23, NO38, or numatin) is an abundant nuclear phosphoprotein that is involved in diverse cellular activities such as ribosome biogenesis and histone assembly. It is frequently overexpressed in proliferating cells and has been regarded as a tumor marker for several human cancers. In some cancers, the NPM1 gene is translocated, mutated, or deleted. This makes it an appealing target for cancer therapy. To better understand the molecular mechanism of NPM1, we have determined the crystal structure of its N-terminal ‘core’ domain (Met9-Asp122). Each monomer folds into an eight-stranded β-barrel with a jellyroll topology. Two pentamers associate in a head-to-head fashion to form a decamer. Our structure of human NPM-core reveals significant deviations in the relative orientation of the two pentameric rings from the decamers of Xenopus NO38-core and Xenopus nucleophosmin-core. This structural difference, which is hardly predictable by homology modeling, is likely to have a significant implication for the histone chaperone function of NPM.

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**Crystal structure of D-Erythronate-4-phosphate dehydrogenase complexed with NAD**

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Pyridoxal-5’-phosphate (the active form of vitamin B6) is an essential cofactor in many enzymatic reactions. While animals lack any of the pathways for de novo synthesis and salvage of vitamin B6, it is synthesized by two distinct biosynthetic routes in bacteria, fungi, parasites, and plants. One of them is the Pdx/PdxU pathway found in the g subdivision of proteobacteria. It depends on the pdxB gene, which encodes erythronate-4-phosphate dehydrogenase (PdxB), a member of the D-isomer specific 2-hydroxycarboxylic dehydrogenase superfamily. Although three-dimensional structures of other functionally related dehydrogenases are available, no structure of PdxB has been provided. To provide the missing structural information and to gain insights into the catalytic mechanism, we have determined the first crystal structure of erythronate-4-phosphate dehydrogenase from Pseudomonas aeruginosa in the ligand-bound state. It is a homodimeric enzyme consisting of 380-residue subunits. Each subunit consists of three structural domains: the lid domain, the nucleotide-binding domain, and the C-terminal dimerization domain. The latter domain has a unique fold and is largely responsible for dimerization. Interestingly, two subunits of the dimeric enzyme are bound with different combinations of ligands in the crystal and they display significantly different conformations. Subunit A is bound with NAD and a phosphate ion, while subunit B, with a more open active site cleft, is bound with NAD and L(-)-tartrate. Our structural data allows a detailed understanding of cofactor and substrate recognition, thus providing substantial insights into PdxB catalysis.

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**Crystal structure of CipP from Bacillus subtilis at 2.4 Å resolution and the characterization of its proteolytic activity**

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An ATP-dependent chaperone/protectase complex, CipXP plays an important role in the protein degradation in most bacteria or in mitochondria and chloroplast of eukaryotes. CipXP consists of two different components, CipP and CipX. CipP is a serine protease that has 14 identical subunits organized in two stacked heptameric rings where CipP is a homotetrameric AAA-ATPase that binds, denatures, and translocates protein substrates into proteolytic chamber. We have determined the crystal structure of CipP from Bacillus subtilis (BsP) at 2.4 Å resolution and tested proteolytic activity against their peptide and protein substrates in presence of CipX. This refined model shows a similarity with previously solved structures of CipP from other species including E. coli CipP (EcP). Although the structural and sequential resemblance between BsP and EcP is significantly high, CipP from E. coli is not able to stimulate the proteolytic activity of BsP and CipP from B. subtilis is also not able to stimulate that of EcP. It is stark different to HslVU, a similar bacterial ATP-dependent protease, which shows mutual activation through swapped combinations.